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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/590,896	USHIJIMA ET AL.	
	Examiner	Art Unit	
	OLUWATOSIN OGUNBIYI	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 July 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-8,14-17 and 23-45* is/are pending in the application.

4a) Of the above claim(s) *1-7 and 26-45* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *8, 14-17 and 23-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continued Examination Under 37 CFR 1.114

1 A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 7/14/10 has been entered.

2 Claims 9-13 and 18-22 have been cancelled. Claims 1-8, 14-17 and 23-45 are pending. Claims 1-7 and 26-45 are withdrawn further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 4/22/09. Claims 8, 14-17 and 23-25 are under examination.

Claim Objections-Withdrawn

3 The objection to claims 15 and 24 under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim is withdrawn in view of the amendment to the claims 15 and 24.

Claim Rejections -Withdrawn

4. The rejection of claims 8, 15-17, 24 and 25 under 35 U.S.C. 102(b) as being anticipated by Fischetti et al. WO 00/47744 August 17, 2000 is withdrawn in view of the amendment to the claims.

Claim Rejections -Maintained

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it

pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. The rejection of claims 8, 14-17 and 23-25 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention is maintained. **This is a written description rejection.**

Independent claims 8 and 17 and dependent claims are drawn to:

an isolated variant of an *Erysipelothrix rhusiopathiae* surface protective antigen SpaA protein or

of a shortened form thereof (known as ΔSpaA protein), which is a shortened form of the SpaA protein in which a portion of the SpaA protein is deleted, wherein said variant is immunogenic, and expressed in *E. coli* as inclusion bodies and has an amino acid sequence with an amino acid substitution consisting of one or a combination of more than one selected from the group consisting of (1) to (7) as described below:

(1) the 69th amino acid from the N-terminal
encompassing the signal sequence is substituted with glycine;

(2) the 154th amino acid from the N-terminal
encompassing the signal sequence is substituted with glycine;

(3) the 203rd amino acid from the N-terminal
encompassing the signal sequence is substituted with threonine;

(4) the 214th amino acid from the N-terminal
encompassing the signal sequence Is substituted with glutamine;

(5) the 253rd amino acid from the N-terminal
encompassing the signal sequence is substituted with threonine;

(6) the 278th amino acid from the N-terminal
encompassing the signal sequence substituted with glycine;
and

(7) the 531st amino acid from the N-terminal
encompassing the signal sequence is substituted with glycine.

Claim 15 is drawn to the isolated variant of claim 8 or 14, wherein the SpaA protein and the ΔSpaA protein has an amino acid sequence as depicted in SEQ ID NO: 2 or the sequence as depicted in SEQ ID NO: 2 with a deletion at the C-terminal, respectively, wherein the amino acid substitution is introduced.

Claim 24 is drawn to the composition of claim 17 or 23, wherein the SpaA protein and the ΔSpaA protein has an amino acid sequence as depicted in SEQ ID NO: 2 or the sequence as depicted in SEQ ID NO: 2, with a deletion at the C-terminal, respectively, wherein the amino acid substitution is introduced.

Written Description Analysis

The claims are drawn to any variant of any SpaA protein isolated from any type or strain of *Erysipelothrix rhusiopathiae* or a shortened from of any SpaA protein isolated from any type or strain of *Erysipelothrix rhusiopathiae* in which any portion of the any SpaA protein is deleted. Claim 8 does not identify the SpaA protein by any structure or SEQ ID NO:, thus is drawn to any SpaA protein isolated from any type or strain of *Erysipelothrix rhusiopathiae*. Furthermore, the variant of any SpaA protein isolated from any type or strain of *Erysipelothrix rhusiopathiae* or a shortened from of any SpaA protein isolated from any type or strain of *Erysipelothrix rhusiopathiae* in which any portion of the any SpaA protein is deleted has an amino acid substitution of one or a combination of more than one residues as set forth in (1) to (7). Thus, the scope of the claims comprises:

(1) any variant of any SpaA protein isolated from any type or strain of *Erysipelothrix rhusiopathiae* that has an amino acid substitution of one or a combination of more than one residues as set forth in (1) to (7). This is a large and variant genus because it covers any SpaA

protein isolated from any type or strain of *Erysipelothrix rhusiopathiae* and the substitutions occur at the particular positions of the amino acid sequence of any SpaA protein isolated from any type or strain of *Erysipelothrix rhusiopathiae*. No amino acid sequence of the SpaA protein is disclosed in the claim and as evidenced by the specification there are different strains and serotypes of *Erysipelothrix rhusiopathiae* (see specification p. 2 lines 1-9, see claim 16) and all of these would have different SpaA proteins (see p. 3 lines 20-25 and p. 4) Makino et al (Microbial Pathogenesis 1998; 25:101-109, cited in IDS) teach that there are 21 strains of *Erysipelothrix rhusiopathiae* and that there is some degree of polymorphism in the SpaA gene among the different strains. See p. 103 column 1 first incomplete paragraph and p. 104 table 1.

(2) any variant of any SpaA protein isolated from any type or strain of *Erysipelothrix rhusiopathiae* that has any portion deleted that further comprises an amino acid substitution of one or a combination of more than one residues as set forth in (1) to (7). This is a large and variant genus comprising any SpaA protein isolated from any type or strain of *Erysipelothrix rhusiopathiae* that have deletions anywhere in the protein sequence and further comprising an amino acid substitution of one or a combination of more than one residues as set forth in (1) to (7).

The scope of the claim includes numerous structural variants and the genus is highly variant because a significant number of structural differences between genus members is permitted.

The claims require the following functions: that the members of the genus be immunogenic and when expressed in *E.coli* the members of the genus form inclusion bodies.

The scope of claims 15 and 24 are drawn to the SpaA protein or fragments thereof (i.e. has **an** amino acid sequence as depicted in SEQ ID NO: 2) comprising the amino acid substitutions listed in claims 8 or 14 or the ΔSpaA protein which has the sequence as depicted in SEQ ID NO; 2 with a deletion anywhere in the C-terminal and comprising the amino acid substitutions listed in claims 8 or 14. The scope of the claim includes numerous structural variants and the genus is highly variant because a significant number of structural differences between genus members is permitted.

Actual Reduction to Practice

The full length SpaA gene is encoded by the nucleotide sequence from the 79th to 1881st. See p. 30-31, example 1.

As to a shortened form of SpaA known as ΔSpaA protein, the specification teaches cloning of this shortened form of SpaA from type 1 Fujisawa strain and Koganai strain and type 2 Tama 96 strain and SE9 strain wherein the shortened from of SpaA is encoded by a partial SpaA gene up till the 1260th nucleotide and codes for a shortened form of SpaA protein (with deletion of 207 amino acid residues at the C-terminal). See p. 30-31 example 1.

The specification proceeds to determine whether inclusion bodies of SpaA and ΔSpaA protein form inclusion bodies and it was determined that out of all of the ΔSpaA from the Fujisawa strain, Koganai strain, Tama 96 strain, SE9 strain only *E. coli* expressing ΔSpaA from the SE-9 strain formed inclusion bodies in *E. coli*. Also, for the full length SpaA protein, only *E. coli* expressing full length SpaA from the SE-9 strain was determined to form inclusion bodies in *E. coli*. See p. 34 table 1.

Table 1

	Clones forming inclusion bodies/Clones expressing ΔSpaA	Clones forming inclusion bodies/Clones expressing SpaA
Fujisawa strain (type 1)	0/3	ND
SE-9 strain (type 2)	3/30	1/15
Tama 96 strain (type 2)	0/3	ND
Koganai strain (type 1)	0/3	ND

For the four *E. coli* clones expressing SpaA and ΔSpaA of the SE 9 strain, plasmids were extracted and the amino acid substitutions were discovered as compared with wild type sequence of the SE9 strain (i.e. SEQ ID NO: 7) as set forth in table 2 p. 34.

Table 2

Nucl. position	Nucleotide substitution (corresponding amino acid substitution)	Clone
206th	A to G (the 69th glutamic acid to glycine)	No. 2
461st	A to G (the 154th glutamic acid to glycine)	No. 2
608th	T to C (the 203rd isoleucine to threonine)	No. 2
642nd	T to G (the 214th histidine to glutamine)	No. 1
758th	T to C (the 253rd methionine to threonine)	No. 1
833rd	A to G (the 278th aspartic acid to glycine)	No. 3
1591st	A to G (the 531st arginine to glycine)	No. 4

The discovered ability of the amino acid substitutions to form inclusion bodies, was confirmed by replacing portions of the SpaA and ΔSpaA of the SE 9 strain wild type sequence with corresponding portions harboring the amino acid substitution and transforming *E.coli* to determine that inclusion bodies were formed. See example 2, p. 35-41.

Of the SE9 strain SpaA and ΔSpaA protein harboring amino acid substitutions wherein the SpaA and ΔSpaA protein substitution mutants formed inclusion bodies in *E. coli*, the specification reduced to practice the immunogenicity of the following in table 3:

Table 3

Purified protein	ΔSpaA			SpaA	
Site of subst. in SpaA gene	642nd 758th	206th 461st 608th	833rd	1591st	642nd 758th
Protein conc. (mg/ml)	2.30	1.91	2.33	2.11	2.28
Fold of dilution	No. of survival /No. of challenged	No. of survival /No. of challenged	No. of survival /No. of challenged	No. of survival /No. of challenged	No. of survival /No. of challenged
625	10/10	10/10	10/10	10/10	10/10
3125	9/10	8/10	10/10	10/10	10/10
15625	5/10	0/10	4/10	4/10	6/10
78125	0/10	0/10	0/10	0/10	0/10
Median protective dose in mice (ug)	0.0864	0.1885	0.0875	0.0793	0.0621

Therefore, the specification reduced to practice at the time of filing :

(1) The SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at position 531 i.e. 531st arginine to glycine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(2) The SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at both of position 214 i.e.214st histidine to glutamine and 253rd methionine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(3) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising both amino acid substitutions 214th histidine to glutamine and 253rd methionine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(4) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising all three amino acid substitutions comprising the 69th glutamic acid to glycine and 154th glutamic acid to glycine and 203rd isoleucine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(5) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising the amino acid substitution the 278th aspartic acid to glycine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

Sufficient Relevant Identifying Characteristics

The specification does not describe other members of the genus of proteins to which the claims are drawn that forms in inclusion bodies when expressed in *E. coli* and that is immunogenic.

Apart from the SpaA or ΔSpaA protein of the SE9 strain substitution mutants that are expressed as inclusion bodies in *E.coli* and are immunogenic as set forth in table 3, the specification does not describe the amino acid substitution(s) of any other type of SpaA protein whether from any other strains or the amino acid substitution(s) of any deletion mutant of any

other SpaA protein that result in said protein(s) being expressed in *E. coli* as inclusion bodies and that are also immunogenic.

The specification does not disclose complete structure, partial structure, physical or chemical properties of other members of the genus to which the claims are drawn that form inclusion bodies and that are immunogenic.

The specification does not describe the structure common to all the members of the genus of SpaA or ΔSpaA protein variants that results in the function or characteristics of being expressed in *E. coli* as inclusion bodies and that are also immunogenic.

Method of making the claimed invention and Predictability in the art

The specification teaches that any SpaA protein from any strain of *Erysipelothrix rhusiopathiae* can be used to make the instant invention. However, only the four *E. coli* clones expressing SpaA and ΔSpaA of the SE 9 strain were found to form inclusion bodies when expressed in *E.coli* as set forth in table 1.

Table 1

	Clones forming inclusion bodies/Clones expressing ΔSpaA	Clones forming inclusion bodies/Clones expressing SpaA
Fujisawa strain (type 1)	0/3	ND
SE-9 strain (type 2)	3/30	1/15
Tama 96 strain (type 2)	0/3	ND
Koganai strain (type 1)	0/3	ND

As evidenced by table 1, it is unpredictable that other SpaA proteins or deletion variants of SpaA protein can form inclusion bodies when expressed in *E. coli*, as evidenced by table 1.

Furthermore, as to the genus of SpaA proteins that comprise a deletion in any portion and further comprise one or combination of the amino acid substitutions listed in the claims, the specification does not describe the common structure i.e. the immunoepitope(s) of said genus that correlates with function i.e. immunogenicity so that one of skill in the art can envision

which amino acids or combinations of amino acids can be deleted in a Spa A protein and ΔSpaA in addition to the listed amino acid substitutions and still retain immunogenicity.

Colman et al. (Research in Immunology 145: 33-36, 1994, p.33 column 2, p. 35 column 1) disclose that a single amino acid change in an antigen can effectively abolish the interaction with an antibody. This underlies the importance of the description of the immunoepitope(s) and which amino acid deletion(s) and where and coupled with the instant amino acid substitutions in the deletion variant still retains immunogenicity. Houghten et al. (New Approaches to Immunization, Vaccines 86, Cold Spring Harbor Laboratory, p. 21-25, 1986) taught the criticality of individual amino acid residues and their positions in peptide antigen-antibody interactions. Houghten et al state (see page 24): "One could expect point mutations in the protein antigen to cause varying degrees of loss of protection, depending on the relative importance of the binding interaction of the altered residue. A protein having multiple antigenic sites, multiple point mutations, or accumulated point mutations at key residues could create a new antigen that is precipitously or progressively unrecognizable by any of the antibodies in the polyclonal pool."

Applicants clearly did not provide written description of the common structure contained in the *E. rhusiopathiae* SpaA from the different strains or types necessary for immunogenicity so that one of ordinary skill in the art can envision the areas which to delete and not to delete and result in a deletion variant further comprising the instant amino acid substitution(s) and still possess immunogenicity and is expressed as inclusion bodies in *E.coli*.

The disclosure of: 1) The full length SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at position 531 i.e. 531st arginine to glycine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies; (2) The full length SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at both of position 214 i.e.214st histidine to glutamine and 253rd methionine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies; (3) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising both amino acid substitutions 214th histidine to glutamine and 253rd methionine to threonine, that is

immunogenic and when expressed in *E. coli* forms inclusion bodies; (4) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising all three amino acid substitutions comprising the 69th glutamic acid to glycine and 154th glutamic acid to glycine and 203rd isoleucine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies and (5) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising the amino acid substitution the 278th aspartic acid to glycine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies is insufficient to describe the large and variant genus of proteins the scope of which is set forth above. Immunogenicity has to be empirically determined. The art recognizes that defining epitopes is not easy and there is a confusing divergence between the textbook definition of epitope and the definition that is in use in published descriptions of experimental investigations and that epitopes must be empirically determined (Greenspan et al, Nature Biotechnology 17:936-937, 1999).

In such an unpredictable art of protein mutation and the effect on antigenicity or immunogenicity as set forth supra, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus. In the instant case, the specification only discloses that the SpaA and ΔSpaA proteins of the SE 9 strain as set forth in table 1 formed inclusion bodies in E.coli and with the particular amino acid substitutions were immunogenic. See *Noelle v Lederman*. 355 F. 3d 1343, 1350, 69 USPQ2d 1508, 1514 (*Fed. Cir. 2004*) and *In re Alonso* (Fed. Cir. 2008-1079). The specification has not reduced to practive any other SpaA and ΔSpaA protein from any other strain of *E. rhushiopathie* that has one or more of the listed amino acid substitutions that results in a protein that forms inclusion bodies in *E. coli* and is immunogenic.

The fact that one could screen for which variants are expressed as inclusion bodies in *E.coli* and are immunogenic is not the standard for written description. The written description requirement is separate and distinct from the enablement requirement (See also *Univ. of Rochester v. G.D. Searle & Co*., 358 F.3d 916, 920-23, 69 USPQ2d 1886, 1890-93 (Fed. Cir. 2004) and adequate written description requires more than a mere reference to a potential method for identifying candidate polypeptides. The purpose of the written description requirement is broader than to merely explain how to 'make and use' [the invention] *Vas-Cath, Inc. v.*

Mahurkar, 935 F.2d 1555, 1560, 19 USPQ2d 1111, 1114 (Fed. Cir. 1991). Since the specification does not describe the common structure of said genus of protein variants that correlates with function i.e. immunogenicity, the skilled artisan would conclude that Applicants as of the time of filing were not in possession of the genus of variants to which the claims are drawn.

While the general knowledge in the art supplements the written description concerning making deletions and/or amino acid substitutions in proteins, thus every member of the genus of isolated variant SpaA or ΔSpaA proteins can be made, the specification however does not disclose the common structure e.g. immunoepitope(s) of the genus of SpaA proteins comprising the one or a combination of the listed amino acid substitution that results in a variant of SpaA that is expressed as inclusion bodies in *E. coli* and is immunogenic and also immunoepitopes of the genus of ΔSpaA proteins comprising the one or a combination of the listed amino acid substitution that results in a variant of ΔSpaA that is expressed as inclusion bodies in *E. coli* and is immunogenic.

In view of the above, the claimed subject matter is not supported by an adequate written description because a representative number of species that are immunogenic and are expressed as inclusion bodies in *E.coli* has not been described. Thus, Applicants as of the time of filing were only in possession of as set forth in table 3.

1) The SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at position 531 i.e. 531st arginine to glycine, that is immunogenic and when expressed in E. coli forms inclusion bodies.

(2) The SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at both of position 214 i.e.214st histidine to glutamine and 253rd methionine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(3) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising both amino acid substitutions 214th histidine to glutamine and 253rd methionine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(4) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising all three amino acid substitutions comprising the 69th glutamic acid to glycine and 154th glutamic acid to glycine and 203rd isoleucine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(5) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising the amino acid substitution the 278th aspartic acid to glycine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

6. Applicants' arguments and the response:

Applicants argue:

In the instant case, the claimed variant of SpaA protein and its shortened form, ΔSpaA protein, according to main claims 8 and 17 of the present application, are defined as an insoluble protein mutated from a soluble Erysipelothrix rhusiopathiae surface protective antigen SpaA or ΔSpaA protein by a specific amino acid substitution, and as a result have a property of being expressed as insoluble inclusion bodies to thereby facilitate recovery and purification of said protein. It should be noted that this property of being expressed as insoluble inclusion bodies is only possible when the specific amino acid substitution(s) as defined in the amended claims is/are introduced. This is fully described in the disclosure see for instance, paragraphs [0024] to [0033]. For example, paragraph [0033] describes that the amino acid sequence of SpaA or ΔSpaA protein may be the sequence as depicted in SEQ ID NO: 2 or the sequence as depicted in SEQ ID NO: 2 with deletion at its C-terminal, respectively, and it is this sequence in which the desired amino acid substitution may be introduced. This clearly provides for a relevant identifying characteristics, such as structure (i.e., the structure of the amino acid sequence).

Applicants' argument is carefully considered but is not found persuasive. Mere description in the claims of how to make the claimed variant is insufficient to put Applicants in possession of the genus of claimed variants that are immunogenic and are expressed as inclusion bodies in *E.coli*. The written description requirement is separate and distinct from the enablement requirement (See also *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 920-23, 69 USPQ2d 1886, 1890-93 (Fed. Cir. 2004) and adequate written description requires more than a mere reference to a potential method for identifying candidate polypeptides. The purpose of the written description requirement is broader than to merely explain how to 'make and use' [the invention] *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1560, 19 USPQ2d 1111, 1114 (Fed.

Cir. 1991). Applicants' argument that the amino acid sequence of SpaA or ΔSpaA protein may be the sequence as depicted in SEQ ID NO: 2 or the sequence as depicted in SEQ ID NO: 2 with deletion at its C-terminal, respectively, and it is this sequence in which the desired amino acid substitution may be introduced and that this clearly provides for a relevant identifying characteristics, such as structure (i.e., the structure of the amino acid sequence) is not persuasive. The claims are drawn to a large and variant genus of isolated variants of Spa and ΔSpaA from the different strains of SpaA. There are 21 strains of *Erysipelothrix rhusiopathiae* and there is polymorphism amongst the strain in their SpaA. The scope of claims 8, 14, 16, and 17, 23, 25 is drawn to any of these SpaA genes and their shortened forms thereof and not only limited to SEQ ID NO: 2 which is the Spa protein of fujisawa strain. The scope of claims 15 and 24 are drawn to the SpaA protein or fragments thereof (i.e. has **an** amino acid sequence as depicted in SEQ ID NO: 2) comprising the amino acid substitutions listed in claims 8 or 14 or the ΔSpaA protein which has the sequence as depicted in SEQ ID NO; 2 with a deletion anywhere in the C-terminal and comprising the amino acid substitutions listed in claims 8 or 14. The scope of the claims 15 and 24 includes numerous structural variants and the genus is highly variant because a significant number of structural differences between genus members is permitted. Also, any deletion can be made at the C terminal. There is no reduction to practice of any SpaA or ΔSpaA protein with the sequence depicted in SEQ ID NO: 2 or the sequence as depicted in SEQ ID NO: 2 with deletion anywhere at its C-terminal that results in a protein that is immunogenic and is expressed as inclusion bodies in *E.coli*. As set forth in the rejection above, Houghten et al state (see page 24): "One could expect point mutations in the protein antigen to cause varying degrees of loss of protection, depending on the relative importance of the binding interaction of the altered residue. A protein having multiple antigenic sites, multiple point mutations, or accumulated point mutations at key residues could create a new antigen that is precipitously or progressively unrecognizable by any of the antibodies in the polyclonal pool. In the instant case, Applicants as of the time of filing were only in possession of the variant SpaA or ΔSpaA protein of the SE9 strain with the particular amino acid subsitutitions as set forth in table 3 that are immunogenic and form inclusion bodies in E. *coli.*

Furthermore, Applicants argument that contrary to the examiner's position, the claims do not relate to any number of amino acid(s) at any location of the SpaA protein can be deleted, is not found persuasive. Claims 8, 14, 16, 17, 23 and 25, are drawn to any ΔSpaA protein wherein any portion is deleted. Furthermore, claims 15 and 24 are drawn to any ΔSpaA protein with any deletion in the C terminal. The claims are drawn to a large genus of ΔSpaA protein comprising members with different structural features because any SpaA protein derived from any of the 21 strains of *Erysipelothrix rhusiopathiae* can be used to make members of the genus and each SpaA protein of a particular strain can comprise a deletion in any portion or a deletion in any part of the C-terminal and the claims require that the genus of SpaA or ΔSpaA with the one or more of the listed amino acid substitutions be immunogenic and are expressed as inclusion bodies in *E. coli*. "A deletion in any portion or a deletion in any part of the C-terminal" results in a plethora of different deletion mutants that can have 1,2,3,4,5 etc amino acids deleted anywhere in the SpaA protein or anywhere in the C-terminal. Furthermore, the genus is highly variant because in addition to the numerous possibilities of deletions, the deletion variants further comprise one or a combination of more than one of the amino acid substitutions recited in the claims. As set forth above, the specification does not set forth the common structure i.e. the immunoepitope(s) of said genus of deletion and substitution variants that correlates with function i.e. expressed as inclusion bodies in *E. coli* **and** immunogenicity and so that one of skill in the art can envision which amino acids or combinations of amino acids can be deleted in a SpaA protein and ΔSpaA protein and still retain immunogenicity. There is no sufficient description of the common structure of the members of this genus that correlates with immunogenicity of the proteins. Even if the amino acid substitutions that result in expression as inclusion bodies in *E. coli* are described for all the Spa proteins from all the different 21 strains of *E. rhusiopathie*, the common structure of the members of the instant genus of SpaA protein and ΔSpaA protein derived from the numerous strains of *Erysipelothrix rhusiopathiae* that correlates with immunogenicity is not described at the time of filing, so that one of skill in the art would be able to envision the portions which can or cannot be deleted and in addition to the amino acid substitutions still maintain immunogenicity. It is not even clear that for the 21 strains of *Erysipelothrix rhusiopathiae* that all their SpaA proteins have the recited amino acid residues at the recited positions. As evidenced by Makino et al, there is some degree of restriction

fragment polymorphism among *Erysipelothrix rhusiopathiae* strains, thus it is unlikely that all the SpaA proteins derived from all 21 strains of *Erysipelothrix rhusiopathiae* will have the same exact amino acid sequence, and the instant specification provides no evidence to the contrary.

Applicants' arguments focus on the fact the specification clearly teaches that the property of being expressed as insoluble inclusion bodies is only possible when one or more of the above noted specific amino acid substitution(s) are introduced. Applicants are pointed to the claims in that another function is listed i.e. the proteins have to be immunogenic. As stated above, there is no sufficient description of the common structure of the members of the genus of isolated variants of SpaA protein and ΔSpaA protein that correlates with immunogenicity of the proteins.

Applicants argument that the specification, at for example, paragraph [0067], teaches a ΔSpaA protein encoded by a partial SpaA gene up till the 1260th nucleotide and codes for a shortened form of SpaA protein (with deletion of 207 amino acid residues at the C-terminal with particular amino acid substitutions at particular positions, and that this was found to be immunogenic, is not persuasive. The specification teaches that any SpaA protein from any strain of *Erysipelothrix rhusiopathiae* can be used to make the instant invention. However, only the four *E. coli* clones expressing SpaA and ΔSpaA of the SE 9 strain were found to form inclusion bodies when expressed in *E.coli* as set forth in table 1.

Table 1

	Clones forming inclusion bodies/Clones expressing ΔSpaA	Clones forming inclusion bodies/Clones expressing SpaA
Fujisawa strain (type 1)	0/3	ND
SE-9 strain (type 2)	3/30	1/15
Tama 96 strain (type 2)	0/3	ND
Koganai strain (type 1)	0/3	ND

For the four *E. coli* clones expressing SpaA and ΔSpaA of the SE 9 strain that formed inclusion bodies, plasmids were extracted and amino acid substitutions were discovered as compared with wild type sequence of the SE9 strain (i.e. SEQ ID NO: 7) as set forth in table 2 p. 34.

Table 2

Nucl. position	Nucleotide substitution (corresponding amino acid substitution)	Clone
206th	A to G (the 69th glutamic acid to glycine)	No. 2
461st	A to G (the 154th glutamic acid to glycine)	No. 2
608th	T to C (the 203rd isoleucine to threonine)	No. 2
642nd	T to G (the 214th histidine to glutamine)	No. 1
758th	T to C (the 253rd methionine to threonine)	No. 1
833rd	A to G (the 278th aspartic acid to glycine)	No. 3
1591st	A to G (the 531st arginine to glycine)	No. 4

The discovered ability of the amino acid substitutions to form inclusion bodies, was confirmed by replacing portions of the SpaA and ΔSpaA of the SE 9 strain wild type sequence with corresponding portions harboring the amino acid substitution and transforming *E.coli* to determine that inclusion bodies were formed. See example 2, p. 35-41.

Of the SE9 strain SpaA and ΔSpaA protein harboring amino acid substitutions wherein the SpaA and ΔSpaA protein substitution mutants form inclusion bodies in *E. coli*, the specification reduced to practice the immunogenicity of the following in table 3:

Table 3

Purified protein	ΔSpaA			SpaA	
Site of subst. in SpaA gene	642nd 758th	206th 461st 608th	833rd	159lst	642nd 758th
Protein conc. (mg/ml)	2.30	1.91	2.33	2.11	2.28
Fold of dilution	No. of survival /No. of challenged	No. of survival /No. of challenged	No. of survival /No. of challenged	No. of survival /No. of challenged	No. of survival /No. of challenged
625	10/10	10/10	10/10	10/10	10/10
3125	9/10	8/10	10/10	10/10	10/10
15625	5/10	0/10	4/10	4/10	6/10
78125	0/10	0/10	0/10	0/10	0/10
Median protective dose in mice (ug)	0.0864	0.1885	0.0875	0.0793	0.0621

No other isolated variant of the SpaA protein or isolated variant of the ΔSpaA protein comprising one or more of the amino acid substitution that is immunogenic and expressed as inclusion bodies in *E. coli* was reduced to practice apart from those in table 3 and these are isolated variants from the SE9 strain of *Erysipelothrix rhusiopathiae*.

Applicants' argument that in addition, the specification at paragraphs [0073] and [0074] teaches that the region and size of ΔSpaA protein, obtained by deletion of a portion of SpaA protein, is not subject to restriction in so far as ΔSpaA protein remains immunogenic and, when amino acid substitution is introduced, is capable of forming inclusion bodies, is not persuasive. Applicants statement is in agreement with the scope of the claims in that any portion of the SpaA protein can be deleted ("the region and size of ΔSpaA protein, obtained by deletion of a portion of SpaA protein, is not subject to restriction"), however, of the different SpaA proteins obtained from 21 strains of *Erysipelothrix rhusiopathiae*, the specification does not describe the common structure of the genus of deletion and substitution variants of SpaA and ΔSpaA proteins that is responsible for immunogenicity.

Arguments that the specification even teaches that the ΔSpaA protein can have at least about 1/3 of the C-terminal of SpaA protein deleted and still be used in the present invention, is not persuasive because there are different SpaA from 21 different strains of *Erysipelothrix rhusiopathiae*, the claims do not recite which 1/3 of the C-terminal i.e. particular residues can be deleted, the specification does not describe whether these 1/3 c-terminal residues are present in all SpaA proteins from all strain of *Erysipelothrix rhusiopathiae*, and if so, the specification has not described and reduced to practice a representative number of SpaA proteins from the different strains of SpaA proteins that comprise the same deletion in a defined set of C-terminal amino acid residues and the one or combination of amino acid substitutions that result in a protein that is still immunogenic and is expressed as inclusion bodies in *E. coli*. The knowledge in the art teaches that multiple changes in the amino acid sequence in a protein can result in a protein that is no longer immunogenic. Houghten et al state (see page 24): "One could expect point mutations in the protein antigen to cause varying degrees of loss of protection, depending on the relative importance of the binding interaction of the altered residue.

Applicants arguments that specification also indicates that preferably, the ΔSpaA protein comprises 420 amino acid residues from the N-terminal encompassing the signal sequence with deletion of 207 amino acids at the C- terminal, this is not found persuasive because, this preference is not recited in the claims. Furthermore, the specification does not correlate any common structure of the members of the genus of isolated variants of SpaA and ΔSpaA protein derived from all strains of *Erysipelothrix rhusiopathiae* with function i.e. immunogenic and expression in *E. coli* as inclusion bodies.

Even though the disclosure may describe where and to what extent the deletion of the SpaA protein should be in order to obtain the ΔSpaA protein of the claims, there is no correlation of the common structure of the member of the genus of isolated variants of SpaA and ΔSpaA protein derived from all strains of *Erysipelothrix rhusiopathiae* with function i.e. immunogenic and expression in *E. coli* as inclusion bodies.

Applicants statement at the bottom of p. 6 of arguments that the present invention is not drawn to a broad genus of proteins in which any portion of the SpaA protein is deleted and which

is immunogenic and also comprising the recited amino acid substitution is in contradiction to Applicants statement at the beginning of p. 6 that that the region and size of ΔSpaA protein, obtained by deletion of a portion of SpaA protein, is not subject to restriction in so far as ΔSpaA protein remains immunogenic and, when amino acid substitution is introduced, is capable of forming inclusion bodies.

Applicants' argument that the specification clearly identifies the location and amount of the deletions which are required (up to 1/3 of the C- terminal of SpaA protein), as well as, the specific amino acid sequence of the SpaA protein (SEQ ID NO: 2) and it identifies specific mutations in this sequence, is not persuasive. Claims 8, 14, 16, and 17, 23, 25 do not identify the location of the deletion but recites " a portion of the SpaA protein is deleted", claims 8, 14, 16, and 17, 23, 25 do not recite the specific amino acid sequence of the SpaA protein (SEQ ID NO: 2) and claims 8, 14, 16, and 17, 23, 25 do not recite specific mutations in this sequence, claims 15 and 24 recite SEQ ID NO: 2 but do not recite which particular C-terminal amino acid residues of this sequence is deleted and the specification does not reduce to practice any isolated variant of SEQ ID NO: 2 with amino acid substitutions or with deletions as well as amino acid substitutions that result in a protein that is immunogenic and is expressed as inclusion bodies in *E. coli.* SEQ ID NO: 2 is the full length sequence of SpaA protein from Fujisawa strain of *Erysipelothrix rhusiopathiae*. See specification p. 21 lines 6-10. However, the specification only reduced to practice isolated variant of SpaA protein and ΔSpaA derived from the SE9 strain of *Erysipelothrix rhusiopathiae* wherein said variants are immunogenic and expressed as inclusion bodies in *E. coli* as set forth in table 3.

In view of the above, the claimed subject matter is not supported by an adequate written description because a representative number of species that are immunogenic and are expressed as inclusion bodies in *E.coli* has not been described. Thus, Applicants as of the time of filing were only in possession of the following as set forth in table 3 of the specification:

1) The SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at position 531 i.e. 531st arginine to glycine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(2) The SpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising an amino acid substitution at both of position 214 i.e.214st histidine to glutamine and 253rd methionine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(3) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising both amino acid substitutions 214th histidine to glutamine and 253rd methionine to threonine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

(4) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising all three amino acid substitutions comprising the 69th glutamic acid to glycine and 154th glutamic acid to glycine and 203rd isoleucine to threonine, that is immunogenic and when expressed *in E. coli* forms inclusion bodies.

(5) ΔSpaA protein of the SE9 strain of *Erysipelothrix rhusiopathiae* comprising the amino acid substitution the 278th aspartic acid to glycine, that is immunogenic and when expressed in *E. coli* forms inclusion bodies.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. The rejection of claims 8, 14-17 and 23-25 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is maintained.

The claims recite amino acid substitution(s) at particular positions in SpaA protein or ΔSpaA protein. The recitation of said amino acid substitution(s) at particular positions is vague because there is no sequence of a SpaA protein and ΔSpaA protein disclosed in the claims, so that with disclosure of an amino acid sequence, it will be definite as to where in a SpaA protein the amino acid substitution(s) occur.

Furthermore, in claims 15 and 24, by "a deletion at the C-terminal" does this mean that the C-terminal amino acid i.e. the last amino acid at the C-terminal is deleted? Applicants can clarify this issue by including the particular amino acid residue(s) of the protein that is deleted.

Applicants are respectfully requested to amend the claim so that the metes and bounds of what is being claimed is clear.

8. Applicants' arguments and the response:

The present amendment renders the rejection moot. In particular, the claims, as amended, specify an isolated variant of an Erysipelothrix rhusiopathiae surface protective antigen SpaA protein or of a shortened form thereof (known as ΔSpaA protein), which is a shortened form of the SpaA protein in which a portion of the SpaA protein is deleted, and the amended claims make it clear that both variants have the specifically recited substitutions therein such that both have the specific properties of being immunogenic and being expressed as inclusion bodies. The amended claims makes it clear that the specific substitutions therein and the resultant properties refer to both the variant of the SpaA protein and the variant of the ΔSpaA protein, as is consistent with the disclosure, for example, at paragraph [0013].

Applicants' arguments are carefully considered but are not persuasive. Applicants' arguments did not address the other issues stated in the previous rejection in that the claims recite amino acid substitution(s) at particular positions in SpaA protein or ΔSpaA protein. The recitation of said amino acid substitution(s) at particular positions is vague because there is no sequence of a SpaA protein and ΔSpaA protein disclosed in the claims, so that with disclosure of an amino acid sequence, it will be definite as to where in a SpaA protein the amino acid substitution(s) occur. Furthermore, by "a deletion at the C-terminal" does this mean that the C-terminal amino acid i.e. the last amino acid at the C-terminal is deleted? Applicants can clarify this issue by including the particular amino acid residue(s) of the protein that is deleted.

While Applicants point to the specification, for example at paragraph 13 for clarification. Though understanding the claim language may be aided by explanations contained in the written description, it is important not to import into a claim limitations that are not part of the claim. E-Pass Techs., Inc. v. 3Com Corp., 343 F.3d 1364, 1369, 67 USPQ2d 1947, 1950 (Fed. Cir. 2003) ("Interpretation of descriptive statements in a patent's written description is a difficult task, as an inherent tension exists as to whether a statement is a clear lexicographic definition or a

description of a preferred embodiment. The problem is to interpret claims in view of the specification' without unnecessarily importing limitations from the specification into the claims."); Altiris Inc. v. Symantec Corp., 318 F.3d 1363, 1371, 65 USPQ2d 1865, 1869-70 (Fed. Cir. 2003). See MPEP 2111.01.

Status of Claims

Claims 8, 14-17 and 23-25 are rejected. Claims 1-7 and 26-45 are withdrawn. No claims allowed.

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Any inquiry concerning this communication or earlier communications from the examiner should be directed to Oluwatosin Ogunbiyi whose telephone number is 571-272-9939. The examiner can generally be reached on M-F 8:30 am - 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor Robert Mondesi can be reached at 571-272-0956.

The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

/Oluwatosin Ogunbiyi/
Examiner, Art Unit 1645